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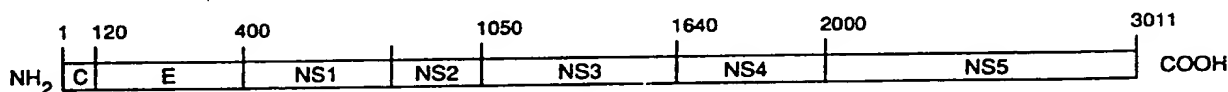
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**(54) Title:** COMBINATIONS OF HEPATITIS C VIRUS (HCV) ANTIGENS FOR USE IN IMMUNOASSAYS FOR ANTI-HCV ANTIBODIES



**(57) Abstract**

Combinations of HCV antigens that have a broader range of immunological reactivity than any single HCV antigen. The combinations consist of an antigen from the C domain of the HCV polyprotein, and at least one additional HCV antigen from either the NS3 domain, the NS4 domain, the S domain, or the NS5 domain, and are in the form of a fusion protein, a simple physical mixture, or the individual antigens commonly bound to a solid matrix.

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COMBINATIONS OF HEPATITIS C VIRUS (HCV) ANTIGENS  
FOR USE IN IMMUNOASSAYS FOR ANTI-HCV ANTIBODIES

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Description

Technical Field

The present invention is in the field of  
15 immunoassays for HCV (previously called Non-A, Non-B  
hepatitis virus). More particularly, it concerns combina-  
tions of HCV antigens that permit broad range immunoassays  
for anti-HCV antibodies.

20 Background

The disease known previously as Non-A, Non-B  
hepatitis (NANBH) was considered to be a transmissible  
disease or family of diseases that were believed to be  
viral-induced, and that were distinguishable from other  
25 forms of viral-associated liver diseases, including that  
caused by the known hepatitis viruses, i.e., hepatitis A  
virus (HAV), hepatitis B virus (HBV), and delta hepatitis  
virus (HDV), as well as the hepatitis induced by  
cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH  
30 was first identified in transfused individuals. Transmis-  
sion from man to chimpanzee and serial passage in  
chimpanzees provided evidence that NANBH was due to a  
transmissible infectious agent or agents. Epidemiologic  
evidence suggested that there may be three types of NANBH:  
35 a water-borne epidemic type; a blood-borne or parenterally  
transmitted type; and a sporadically occurring (community

-2-

acquired) type. However, until recently, no transmissible agent responsible for NANBH had been identified, and clinical diagnosis and identification of NANBH had been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies were agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

In 1987, scientists at Chiron Corporation (the owner of the present application) identified the first nucleic acid definitively linked to blood-borne NANBH. See, e.g., EPO Pub. No. 318,216; Houghton et al., Science 244:359 (1989). These publications describe the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1." HCV is a Flavi-like virus, with an RNA genome.

U.S. Patent Application Serial No. 456,637 (Houghton et al.), incorporated herein by reference, describes the preparation of various recombinant HCV polypeptides by expressing HCV cDNA and the screening of those polypeptides for immunological reactivity with sera from HCV patients. That limited screening showed that at least five of the polypeptides tested were very immunogenic; specifically, those identified as 5-1-1, C100, C33c, CA279a, and CA290a. Of these five polypeptides, 5-1-1 is located in the putative NS4 domain; C100 spans the putative NS3 and NS4 domains; C33c is located within the putative NS3 domain and CA279a and CA290a are located within the putative C domain. The screening also showed

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-3-

that no single polypeptide tested was immunologically reactive with all sera. Thus, improved tests, which react with all or more samples from HCV positive individuals, are desirable.

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#### Disclosure of the Invention

Applicants have carried out additional serological studies on HCV antigens that confirm that no single HCV polypeptide identified to date is immunologically reactive with all sera. This lack of a single polypeptide that is universally reactive with all sera from individuals with HCV may be due, inter alia, to strain-to-strain variation in HCV epitopes, variability in the humoral response from individual-to-individual and/or variation in serology with the state of the disease.

These additional studies have also enabled applicants to identify combinations of HCV antigens that provide more efficient detection of HCV antibodies than any single HCV polypeptide.

Accordingly, one aspect of this invention is a combination of HCV antigens comprising:

- (a) a first HCV antigen from the C domain; and
- (b) at least one additional HCV antigen selected from the group consisting of

- (i) an HCV antigen from the NS3 domain;
- (ii) an HCV antigen from the NS4 domain;
- (iii) an HCV antigen from the S domain;

and

- (iv) an HCV antigen from the NS5 domain.

In one embodiment, the combination of HCV antigens is in the form of a fusion protein comprised of the antigens. In an alternative embodiment, the combination of antigens is in the form of the individual antigens bound to a common solid matrix. In still another embodiment, the combination of antigens is in the form of a mixture of the individual antigens.

-4-

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the above-described combination of HCV antigens under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising contacting said body component with a panel of HCV antigens, simultaneously or sequentially, comprising

- (a) a first HCV antigen from the C domain; and
- (b) at least one additional HCV antigen selected from the group consisting of
  - (i) an HCV antigen from the NS3 domain;
  - (ii) an HCV antigen from the NS4 domain;
  - (iii) an HCV antigen from the S domain;

and

- (iv) an HCV antigen from the NS5 domain under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

Another aspect of the invention is a kit for carrying out an assay for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising in packaged combination

- (a) said combination of HCV antigens;
- (b) standard control reagents; and
- (c) instructions for carrying out the assay.

#### Brief Description of the Drawings

In the drawings:

Figure 1 is the nucleotide sequence of the cDNA sense and anti-sense strand for the HCV polyprotein and the amino acid sequence encoded by the sense strand.

-5-

Figure 2 is a schematic of the amino acid sequence of Figure 1 showing the putative domains of the HCV polypeptide.

## 5 Modes for Carrying Out the Invention

### Definitions

"HCV antigen" intends a polypeptide of at least about 5 amino acids, more usually at least about 8 to 10 amino acids that defines an epitope found in an isolate of HCV. Preferably, the epitope is unique to HCV. When an antigen is designated by an alphanumeric code, the epitope is from the HCV domain specified by the alphanumeric.

"Synthetic" as used to characterize an HCV antigen intends that the HCV antigen has either been isolated from native sources or man-made such as by chemical or recombinant synthesis.

"Domains" intends those segments of the HCV polyprotein shown in Figure 2 which generally correspond to the putative structural and nonstructural proteins of HCV. Domain designations generally follow the convention used to name Flaviviral proteins. The locations of the domains shown in Figure 2 are only approximate. The designations "NS" denotes "nonstructural" domains, while "S" denotes the envelope domain, and "C" denotes the nucleocapsid or core domain.

"Fusion polypeptide" intends a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

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-6-

"Common solid matrix" intends a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

5 "Mammalian body component" intends a fluid or tissue of a mammalian individual (e.g., a human) that commonly contains antibodies produced by the individual. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph  
10 fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Immunologically reactive" means that the antigen in question will react specifically with anti-HCV  
15 antibody commonly present in a significant proportion of sera from individuals infected with HCV.

"Immune complex" intends the combination or aggregate formed when an antibody binds to an epitope on an antigen.

20

#### Combinations of HCV Antigens

Figure 2 shows the putative domains of the HCV polyprotein. The domains from which the antigens used in the combinations derive are: C, S (or E), NS3, NS4, and  
25 NS5. The C domain is believed to define the nucleocapsid protein of HCV. It extends from the N-terminal of the polyprotein to approximately amino acid 120 of Figure 1. The S domain is believed to define the virion envelope protein, and possibly the matrix (M) protein, and is  
30 believed to extend from approximately amino acid 120 to amino acid 400 of Figure 1. The NS3 domain extends from approximately amino acid 1050 to amino acid 1640 and is believed to constitute the viral protease. The NS4 domain extends from the terminus of NS3 to approximately amino  
35 acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about

-7-

amino acid 2000 to the end of the polyprotein and is believed to define the viral polymerase.

The sequence shown in Figure 1 is the sequence of the HCV1 isolate. It is expected that the sequences of other strains of the blood-borne HCV may differ from the sequence of Figure 1, particularly in the envelope (S) and nucleocapsid (C) domains. The use of HCV antigens having such differing sequences is intended to be within the scope of the present invention; provided, however, that the variation does not significantly degrade the immunological reactivity of the antigen to sera from persons infected with HCV.

In general, the HCV antigens will comprise entire or truncated domains, the domain fragments being readily screened for antigenicity by those skilled in the art. The individual HCV antigens used in the combination will preferably comprise the immunodominant portion (i.e., the portion primarily responsible for the immunological reactivity of the polypeptide) of the stated domain. In the case of the C domain it is preferred that the C domain antigen comprise a majority of the entire sequence of the domain. The antigen designated C22 (see Example 4, *infra*), is particularly preferred. The S domain antigen preferably includes the hydrophobic subdomain at the N-terminal end of the domain. This hydrophobic subdomain extends from approximately amino acid 199 to amino acid 328 of Figure 1. The HCV antigen designated S2 (see Example 3, *infra*), is particularly preferred. Sequence downstream of the hydrophobic subdomain may be included in the S domain antigen if desired.

A preferred NS3 domain antigen is the antigen designated C33c. That antigen includes amino acids 1192 to 1457 of Figure 1. A preferred NS4 antigen is C100 which comprises amino acids 1569 to 1931 of Figure 1. A preferred NS5 antigen comprises amino acids 2054 to 2464 of Figure 1.

**SUBSTITUTE SHEET**

-8-

The HCV antigen may be in the form of a polypeptide composed entirely of HCV amino acid sequence or it may contain sequence exogenous to HCV (i.e., it may be in the form of a fusion protein that includes exogenous sequence). In the case of recombinantly produced HCV antigen, producing the antigen as a fusion protein such as with SOD, alpha-factor or ubiquitin (see commonly owned U.S. Pat. No. 4,751,180, U.S. Pat. No. 4,870,008 and U.S. Pat. Application Serial. No. 390,599, filed 7 August 1989, the disclosures of which are incorporated herein, which describe expression of SOD, alpha-factor and ubiquitin fusion proteins) may increase the level of expression and/or increase the water solubility of the antigen. Fusion proteins such as the alpha-factor and ubiquitin fusion are processed by the expression host to remove the heterologous sequence. Alpha-factor is a secretion system, however, while ubiquitin fusions remain in the cytoplasm.

Further, the combination of antigens may be produced as a fusion protein. For instance, a continuous fragment of DNA encoding C22 and C33c may be constructed, cloned into an expression vector and used to express a fusion protein of C22 and C33c. In a similar manner fusion proteins of C22 and C100; C22 and S2; C22 and an NS5 antigen; C22, C33c, and S2; C22, C100 and S2, and C22, C33c, C100, and S2 may be made. Alternative fragments from the exemplified domain may also be used.

#### Preparation of HCV Antigens

The HCV antigens of the invention are preferably produced recombinantly or by known solid phase chemical synthesis. They may, however, also be isolated from dissociated HCV or HCV particles using affinity chromatography techniques employing antibodies to the antigens.

-9-

When produced by recombinant techniques, standard procedures for constructing DNA encoding the antigen, cloning that DNA into expression vectors, transforming host cells such as bacteria, yeast, insect, or mammalian cells, and expressing such DNA to produce the antigen may be employed. As indicated previously, it may be desirable to express the antigen as a fusion protein to enhance expression, facilitate purification, or enhance solubility. Examples of specific procedures for producing representative HCV antigens are described in the Examples, infra, and in parent application Serial No. 456,637.

#### Formulation of Antigens for Use in Immunoassay

The HCV antigens may be combined by producing them in the form of a fusion protein composed of two or more of the antigens, by immobilizing them individually on a common solid matrix, or by physically mixing them. Fusion proteins of the antigen may also be immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface will vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface will be the wall of the well or cup. For assays using beads, the solid surface will be the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface will be the surface of the material from which the dipstick is made. In agglutination assays the solid surface may be the surface of latex or gelatin particles. When individual antigens are bound to the matrix they may be distributed homogeneously on the surface or distributed thereon in a pattern, such as bands so that a pattern of antigen binding may be discerned.

Simple mixtures of the antigens comprise the antigens in any suitable solvent or dispersing medium.

-10-

Assay Formats Using Combinations of Antigens

The HCV antigens may be employed in virtually any assay format that employs a known antigen to detect antibodies. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogenous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech

SUBSTITUTE SHEET

-11-

Immulon™ 1 or Immulon™ 2 microtiter plates or 0.25 inch polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptides is typically washed after  
5 separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

In a homogeneous format, the test sample is incubated with the combination of antigens in solution.  
10 For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

In a standard format, the amount of HCV antibodies forming the antibody-antigen complex is directly  
15 monitored. This may be accomplished by determining whether labeled anti-xenogenic (e.g., anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format,  
20 the amount of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

Complexes formed comprising anti-HCV antibody  
25 (or, in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled HCV antibodies in the complex may be detected using a conjugate of antixenogeneic Ig complexed with a  
30 label, (e.g., an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the HCV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of  
35 precipitate. If no anti-HCV antibody is present in the test specimen, no visible precipitate is formed.

SUBSTITUTE SHEET

-12-

The HCV antigens will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the combination of antigens (either already bound to a solid matrix or separate with reagents for binding them to the matrix), control antibody formulations (positive and/or negative), labeled antibody when the assay format requires same and signal generating reagents (e.g., enzyme substrate) if the label does not generate a signal directly. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay usually will be included in the kit.

The following examples are intended to illustrate the invention and are not intended to limit the invention in any manner.

Example 1: Synthesis of HCV Antigen C33c

HCV antigen C33c contains a sequence from the NS3 domain. Specifically, it includes amino acids 1192-1457 of Figure 1. This antigen was produced in bacteria as a fusion protein with human superoxide dismutase (SOD) as follows. The vector pSODcfl (Steiner et al. (1986), J. Virol. 58:9) was digested to completion with EcoRI and BamHI and the resulting EcoRI, BamHI fragment was ligated to the following linker to form pcflEF:

GATC CTG GAA TTC TGA TAA  
GAC CTT AAG ACT ATT TTA A

A cDNA clone encoding amino acids 1192-1457 and having EcoRI ends was inserted into pcflEF to form pcflEF/C33c. This expression construct was transformed into D1210 E. coli cells.

The transformants were used to express a fusion protein comprised of SOD at the N-terminus and in-frame C33c HCV antigen at the C-terminus. Expression was ac-

-13-

complished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of the transformants. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000 x g at 4°C for 20 minutes. The packed cells can be stored at -80°C for several months.

In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD-C33c was isolated and subjected to differential extraction with an alkaline-NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose and Q-Sepharose.

The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000 x g for 15 min at 4°C. After the supernatant was removed, the cell pellets were resuspended in 10 ml of Buffer A1 (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM beta-mercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4,000 x g for 15 minutes at 4°C. After removal of the clear supernatant (periplasmic fraction I), the cell pellets were resuspended in Buffer A1, incubated on ice for 10 minutes, and again centrifuged at 4,000 x g for 15 minutes at 4°C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5

SUBSTITUTE SHEET



-14-

ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter)(obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

10           The insoluble fraction of the crude extract was collected by centrifugation at 20,000 x g for 15 min at 4°C, washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q water.

15           A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging it 20,000 x g for 20 min at 4°C, and retaining the supernatant.

20           In order to purify SOD-C33c on S-Sepharose, the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose Fast Flow (1.5 x 10 cm) which had been equilibrated with  
25 Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA). After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected. The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order  
30 to identify fractions containing SOD-C33c, aliquots of the fractions were analyzed by electrophoresis on 10% polyacrylamide gels containing SDS followed by staining with Coomassie blue. The fractions are also analyzable by Western blots using an antibody directed against SOD.  
35 Fractions containing SOD-C33c were pooled.

-15-

Further purification of SOD-C33c was on a Q-Sepharose column (1.5 x 5 cm) which was equilibrated with Buffer B. The pooled fractions containing SOD-C33c obtained from chromatography on S-Sepharose was applied to the column. The column was then washed with Buffer B, and eluted with 60 ml of a gradient of 0.0 to 0.4 M NaCl in Buffer B. The flow rate for application, wash, and elution was 1 ml/min; collected fractions were 1 ml. All fractions from the Q-Sepharose column were analyzed as described for the S-Sepharose column. The peak of SOD-C33c eluted from the column at about 0.2 M NaCl.

The SOD-C33c obtained from the Q-Sepharose column was greater than about 90% pure, as judged by analysis on the polyacrylamide SDS gels and immunoblot using a monoclonal antibody directed against human SOD.

#### Example 2: Synthesis of HCV Antigen C100

HCV antigen C100 contains sequences from the NS3 and NS4 domains. Specifically, it includes amino acids 1569-1931 of Figure 1. This antigen was produced in yeast. A cDNA fragment of a 1270 bp encoding the above amino acids and having EcoRI termini was prepared.

The construction of a yeast expression vector in which this fragment was fused directly to the S. cerevisiae ADH2/GAP promoter was accomplished by a protocol which included amplification of the C100 sequence using a PCR method, followed by ligation of the amplified sequence into a cloning vector. After cloning, the C100 sequence was excised, and with a sequence which contained the ADH2/GAP promoter, was ligated to a large fragment of a yeast vector to yield a yeast expression vector.

The PCR amplification of C100 was performed using as template the vector pS3-56<sub>C100m</sub>, which had been linearized by digestion with SalI. pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter

-16-

upstream of the human superoxide dismutase gene, and a downstream alpha factor transcription terminator.

The oligonucleotide primers used for the amplification were designed to facilitate cloning into the expression vector, and to introduce a translation termination codon. Specifically, novel 5'-HindIII and 3'-SalI sites were generated with the PCR oligonucleotides. The oligonucleotide containing the SalI site also encodes the double termination codons, TAA-and TGA. The oligonucleotide containing the HindIII site also contains an untranslated leader sequence derived from the pgap63 gene, situated immediately upstream of the AUG codon. The pEco63GAPDH gene is described by Holland and Holland (1980) and by Kniskern et al. (1986). The PCR primer sequences used for the direct expression of C100m were:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG CTC  
ACT TTC TAT CCC AGA CAA AGC AGA GT 3'

20

and

5' GAG TGC TCG TCG ACT CAT TAG GGG GAA  
ACA TGG TTC CCC CGG GAG GCG AA 3'.

Amplification by PCR, utilizing the primers, and template, was with a Cetus-Perkin-Elmer PCR kit, and was performed according to the manufacturer's directions. The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes; and the final incubation was at 72°C for 10 minutes. The DNA can be stored at 4°C or -20°C overnight.

After amplification, the PCR products were digested with HindIII and SalI. The major product of 1.1 kb was purified by electrophoresis on a gel, and the eluted purified product was ligated with a large SalI-HindIII fragment of pBR322. In order to isolate correct

-17-

recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII-SalI fragments excised from the clones.

5 One of the clones which contained the a HindIII-SalI fragment of the correct size was named pBR322/C100<sup>-</sup>d.

Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-SalI fragment.

The expression vector containing C100 was  
10 constructed by ligating the HindIII-SalI fragment from pBR322/C100<sup>-</sup>d to a 13.1 kb BamHI-SalI fragment of pBS24.1, and a 1369 bp BamHI-HindIII fragment containing the ADH2/GAP promoter. (The latter fragment is described in EPO 164,556). The pBS24.1 vector is described in commonly  
15 owned U.S.S.N. 382,805 filed 19 July 1989. The ADH2/GAP promoter fragment was obtained by digestion of the vector pPGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

Competent HB101 cells were transformed with the  
20 recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and SalI digestion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100<sup>-</sup>d#3.

25 In order to express C100, competent cells of Saccharomyces cerevisiae strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prcl-407[cir-0]) were transformed with the expression vector pC100<sup>-</sup>d#3. The transformed cells were plated on URA-sorbitol, and individual transformants  
30 were then streaked on Leu<sup>-</sup> plates.

Individual clones were cultured in Leu<sup>-</sup>, ura<sup>-</sup> medium with 2% glucose at 30°C for 24-36 hours. One liter of Yeast Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight  
35 culture, and the resulting culture was grown at 30°C at an agitation rate of 400 rpm and an aeration rate of 1 L of

-18-

air per 1 L of medium per minute (i.e., lvvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the  
5 cells were isolated and analyzed for C100 expression.

Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu<sup>-</sup> plates. The cell lysates and crude  
10 extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by Western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364  
15 amino acids. By gel analysis the expressed polypeptide has a MW<sub>r</sub> of 39.9K.

Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These  
20 results suggest that the expressed C100 polypeptide may be insoluble.

### Example 3: Expression of HCV Antigen S2

HCV antigen S2 contains a sequence from the  
25 hydrophobic N-terminus of the S domain. It includes amino acids 199-328 of Figure 1.

The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analogous to that used for the  
30 expression of the C100 polypeptide, described in Example 2.

The template for the PCR reaction was the vector pBR322/Pil4a, which had been linearized by digestion with HindIII. Pil4a is a cDNA clone that encodes amino acids  
35 199-328.

-19-

The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following.

5 For the 5'-region of the S2 sequence:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT  
ACC ACG TCA CCA ATG ATT GCC CTA AC 3';

and

10

for the 3'-region of the S2 sequence:

5' GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC  
ATC ATC ATA TCC CAT GCC AT 3'.

15 The primer for the 5'-region introduces a HindIII site and an ATG start codon into the amplified product. The primer for the 3'-region introduces translation stop codons and a SalI site into the amplified product.

The PCR conditions were 29 cycles of 94°C for a  
20 minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from  
25 pBR322 digested with HindIII and SalI fragment, yielding the plasmid pBR322/S2d.

Ligation of the 413 bp HindIII-SalI S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment  
30 of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing  
35 the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.

-20-

Example 4: Synthesis of HCV C Antigen

HCV antigen C22 is from the C domain. It comprises amino acids 1-122 of Figure 1.

5 The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described supra, except for the following.

10 The template for the PCR reaction was pBR322/Ag30a which had been linearized with HindIII. Ag30 is a cDNA clone that encodes amino acids 1-122. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

15

For the 5'-region of the C sequence:

5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA  
ATC CTA AAC CTC AAA AAA AAA AC 3',

20

and

for the 3'-region of the C sequence:

5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC  
GAC CTA CGC CGG GGG TCT GT 3'.

25

The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a SalI site. The PCR was run for 29 cycles of 94°C for a minute, 37°C  
30 for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the SalI-HindIII large SalI-HindIII fragment of pBR322 yielded  
35 the plasmid pBR322/C2.

-21-

Ligation of the 381 bp HindIII-SalI C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu<sup>-</sup> plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW<sub>r</sub> of approximately 13.6 Kd.

#### 20 Example 5: Synthesis of NS5 Polypeptide

This polypeptide contains sequence from the N-terminus of the NS5 domain. Specifically it includes amino acids 2054 to 2464 of Figure 1. The protocol for the construction of the expression vector encoding the NS5 polypeptide and for its expression were analogous to that used for the expression of C33c (see Example 1).

#### Example 6: Radioimmunoassay (RIA) for Antibodies to HCV

The HCV antigens of Examples 1-5 were tested in an RIA format for their ability to detect antibodies to HCV in the serum of individuals clinically diagnosed as having HCV (Non-A, Non-B) and in serum from blood given by paid blood donors.

The RIA was based upon the procedure of Tsu and Herzenberg (1980) in SELECTED METHODS IN CELLULAR IMMUNOLOGY (W.H. Freeman & Co.), pp. 373-391. Generally,



-22-

microtiter plates (Immulon 2, Removawell strips) are coated with purified HCV antigen. The coated plates are incubated with the serum samples or appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid-phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with  $^{125}\text{I}$ -labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

Specifically, one hundred microliter aliquots containing 0.1 to 0.5 micrograms of the HCV antigen in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the antigen solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The antigens in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Antibody bound to the antigen was determined by the binding of  $^{125}\text{I}$ -labeled  $\text{F}'(\text{ab})_2$  sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were

-23-

added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

Table 1 below presents the results of the tests on the serum from individuals diagnosed as having HCV.

Table 1

	<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
	CVH IVDA	P	P	P(+++)	P	P
	CVH IVDA	P	P	P(+)	P	P
15	CVH IVDA	P	P	P(+)	P	P
	CVH NOS	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
	AVH NOS HS	P	N	N	N	N
	AVH NOS HS	P	N	N	N	N
20	AVH NOS HS	P/N	N	N	N	N
	AVH PTVH	N	N	N	P/N	N
	AVH NOS HS	N	N	N	N	N
	AVH NOS	N	N	N	N	P
	AVH PTVH	N	N	N	N	N
25	AVH IVDA	N	P	N	P	P
	AVH PTVH	P	P/N	N	N	P
	AVH NOS	N	P	N	N	N
	AVH IVDA	N	P	N	P	P
	AVH NOS HS	P/N	N	N	N	N
30	AVH PTVH	N	N	N	N	N
	CVH IVDA	P	P	P	P	P
	CVH IVDA	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
	CVH PTVH	P	P	N	N	N
35	AVH PTVH	P	N	P(+)	P(+++)	N
	CVH PTVH	N	P	P	P	P
	CVH NOS HS	P	P	P	P	N
	CVH NOS	N	P	P/N	P	P

SUBSTITUTE SHEET

-24-

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	CVH IVDA	N	N	N	P    N
	AVH IVDA	P	P	P	P    P
5	AVH IVDA	P	P	P	P    P
	CVH IVDA	P	P	P	P    P
	AVH IVDA	P/N	P	N	P    P
	AVH IVDA	N	P	P	P    N
	CVH PTVH	P	P/N	N	N    N
10	CVH NOS	N	N	N	N    N
	CVH NOS	N	N	N	N    N
	CVH IVDA	P	P	P	P    P
	AVH IVDA	P	P	P	P    P
	CVH PTVH	P	P	P	P    P
15	AVH PTVH?	N	P	P	P    P
	AVH IVDA	N	P	N	P    N
	AVH NOS	N	N	N	N    N
	AVH NOS	N	N	N	N    N
	CVH NOS	N	P	N	N    P
20	CVH NOS	P	P	N	N    N
	CVH NOS HS	P	P	P	P    P
	CVH PTVH	P	P	N	P    P
	AVH nurse	P	P	N	N    N
	AVH IVDA	P	P	P	P    N
25	AVH IVDA	N	P	P(+)	P(+++)    N
	AVH nurse	P/N	P	N	N    N
	AVH PTVH	P/N	P	P	N    P
	AVH NOS	N	P/N	N	N    P
	AVH NOS	N	P	N	P    N
30	AVH PTVH	P	P/N	N	N    N
	AVH PTVH	N	F	N	P    P
	AVH PTVH	P	P	P	P    P
	AVH PTVH	N	F	N	N    P
	CVH PTVH	P/N	P	P(+)	P(+++)    N
35	AVH PTVH	N	P/N	P(+)	P(+++)    P

SUBSTITUTE SHEET

-25-

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	P	(?)	P	N    P
	CVH PTVH	N	P	N	P    P
5	CVH PTVH	N	P	P	P    P
	CVH PTVH	N	N	N	N    N
	AVH NOS	N	N	N	N    N
	AVH nurse	P	P	N	N    N
	CVH PTVH	N	P	N	N    P
10	AVH IVDA	N	P	N	P/N    N
	CVH PTVH	P	P	P(+)	P(+++)    P
	AVH NOS	P	P	N	N    N
	AVH NOS	P/N	P	N	N    P
	AVH PTVH	P/N	P	P	P    P
15	AVH NOS	N	P	P	P    P/N
	AVH IVDA	N	P	N	N    P
	AVH NOS	N	P/N	N	N    N
	AVH NOS	P	P	N	N    P
	AVH PTVH	N	P	P	P    P
20	crypto	P	P	P	P    P
	CVH NOS	N	P	P	P    P
	CVH NOS	N	N	N	N    N
	AVH PTVH	N	P	P(+)	P(++)    N
	AVH PTVH	N	P/N	P(+)	P(++)    P
25	AVH PTVH	N	P/N	P(+)	P(++)    P
	CVH IVDA	P	P	P	P    P
	CVH IVDA	P	P	P	P    P
	CVH IVDA	P	P	P	P    P
	CVH IVDA	P	P	P	P    P
30	AVH NOS	N	P	N	N    N
	CVH IVDA	P	P	P	P    P/N
	AVH IVDA	P	P	P	P    N
	AVH NOS	P	P	N	N    N
	AVH NOS	P	P	N	N    N
35	CVH PTVH	P	P	N	N    P/N

SUBSTITUTE SHEET

-26-

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	N	P	N	P   P
	AVH NOS	N	N	N	N   N
5	AVH NOS	N	P	N	N   N
	AVH NOS	P	N	N	N   N
	CVH NOS	N	N	N	N   N
	AVH NOS	N	P/N	N	N   N
	AVH IVDA	N	P	P	P   P
10	crypto	N	P	N	N   P/N
	crypto	P	P	P/N	P   P
	AVH IVDA	N	N	P	P   N
	AVH IVDA	N	P	P	P   N
	AVH NOS	N	N	N	N   N
15	AVH NOS	N	N	N	N   N
	CVH IVDA	P	P	P	P   P
	CVH PTVH	N	N	N	N   N
	CVH PTVH	P	P	P(+)	P(+++)   P
	CVH PTVH	P	P	P(+)	P(+++)   P
20	CVH NOS	P/N	N	N	N   N
	CVH NOS	N	N	N	N   N
	CVH PTVH	P	P	P	P   P
	CVH PTVH	P	P	P	P   P
	CVH PTVH	P	P	P	P   P
25	AVH IVDA	N	P	P	P   P
	CVH NOS	N	N	N	N   N
	CVH NOS	N	N	N	N   N
	CVH PTVH	P	P	P	P   P
	AVH NOS	P	P	N	N   P/N
30	AVH NOS	N	P/N	N	N   N
	CVH PTVH	P	F	N	N   P
	CVH NOS	N	P/N	N	N   N
	AVH NOS	N	F	N	N   N
	AVH NOS	N	F	N	N   N
35	CVH PTVH	N	P	N	N   N

SUBSTITUTE SHEET

-27-

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH IVDA	N	P	N	P    P
	AVH NOS	P	N	N	N    N
5	CVH NOS	N	N	N	N    N
	CVH NOS	N	N	N	N    N
	CVH IVDA	P	P	P	P    P
	CVH IVDA	P/N	P	P	P    P
	CVH IVDA	P	P	P	P    P
10	CVH IVDA	N	P	P	P    P
	AVH NOS	N	P	N	N    N
	CVH IVDA	N	P	N	N    P
	CVH IVDA	N	P	N	N    P
	AVH PTVH	P	P	N	P    P
15	AVH PTVH	P	P	N	P    P
	CVH NOS	N	P/N	N	N    P/N
	CVH NOS	N	P	N	N    N
	CVH NOS	N	N	N	N    N
	CVH PTVH	P	P	P	P    P
20	CVH PTVH	P	P	P	P    P
	CVH PTVH	P	P	P	P    P
	AVH IVDA	N	P	N	N    P
	AVH IVDA	N	P	P(++)	P(+)    P
	CVH PTVH	P	P	P	P    P
25	AVH PTVH	N	P	P	P    P
	CVH PTVH?	N	P	P	P    P
	CVH PTVH?	P/N	P	P	P    P
	CVH NOS HS	P	P	N	N    N
	CVH IVDA	P	P	P	P    N
30	CVH PTVH	N	P	P	P    P
	CVH PTVH	P	P	P	P    P/N
	CVH NOS	P	P	P	P    P
	CVH IVDA	P	F	F	P    P
	CVH PTVH	P	P	P	P    N
35	CVH PTVH	P	P	P	P    P

SUBSTITUTE SHEET

-28-

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	CVH NOS	N	N	N	N    P/N
	CVH NOS	N	P/N	N	N    P/N
5	CVH PTVH	P	P	P	P    P
	CVH NOS	N	P	N	N    N
	CVH NOS	N	N	N	N    N
	CVH NOS	P	P	N	N    P/N
	CVH NOS	N	N	N	N    N
10	CVH NOS HS	P	P	P	P    P
	CVH NOS HS	P	P	P	P    P
	CVH PTVH	N	N	N	N    N
	AVH PTVH	N	P	P	P    P
	AVH NOS			-	-
15	CVH PTVH	N	P	P/N	P(++)    N
	crypto	P	P	P	P    P
	crypto	P	P	P	P    P
	crypto	N	P	N	N    N
	crypto	N	P	P	P    P
20	CVH PTVH	P	P	P	P    P
	crypto	N	N	N	N    N
	crypto	N	P	N	N    P/N
	crypto	N	P	N	N    P
	crypto	P	P	P	P    P
25	crypto	N	P	N	P    N
	crypto			-	-
	crypto			-	-
	CVH NOS			-	-
	AVH-IVDA	N	P	N	P(+)    P
30					

35

SUBSTITUTE SHEET

-29-

<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
	<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
AVH-IVDA	N	P/N	N	P(++)	N

5

AVH = acute viral hepatitis  
 CVH = chronic viral hepatitis  
 PTVH = post-transfusion viral hepatitis  
 IVDA = intravenous drug abuser  
 crypto = cryptogenic hepatitis  
 NOS = non-obvious source  
 P = positive  
 N = negative

10

15

Per these results, no single antigen reacted with all sera. C22 and C33c were the most reactive and S2 reacted with some sera from some putative acute HCV cases with which no other antigen reacted. Based on these results, the combination of two antigens that would provide the greatest range of detection is C22 and C33c. If one wished to detect a maximum of acute infections, S2 would be included in the combination.

20

Table 2 below presents the results of the testing on the paid blood donors.

25

Table 2

		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
30	1	N	N	N	N	N
	2	N	N	N	N	N
	3	P	P	P	P	P
	4	N	N	N	N	N
	5	N	N	N	N	N
35	6	N	N	N	N	N
	7	N	N	N	N	N
	8	N	N	N	N	N

SUBSTITUTE SHEET



-30-

Antigens					
<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	9	N	N	N	N
	10	N	N	N	N
5	11	N	N	N	N
	12	N	N	N	N
	13	N	N	N	N
	14	N	N	N	N
	15	N	N	N	N
10	16	N	N	N	N
	17	N	N	N	N
	18	P	P	P	P
	19	P	P	N	P
	20	P	P	N	P
15	21	N	N	N	N
	22	N	P	N	P
	23	P	P	P	P
	24	N	N	N	N
	25	N	N	N	N
20	26	N	N	N	N
	27	N	N	N	N
	28	N	N	N	N
	29	N	N	N	N
	30	N	N	N	N
25	31	P	P	N	P
	32	N	N	N	N
	33	N	N	N	N
	34	N	N	N	P
	35	N	N	P	P
30	36	N	N	N	N
	37	N	N	N	N
	38	N	N	N	N
	39	N	N	N	N
	40	N	N	N	N
35	41	N	N	N	P
	42	N	N	N	N

SUBSTITUTE SHEET

-31-

		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	43	N	N	N	N	N
	44	N	N	N	N	N
5	45	N	N	N	N	N
	46	N	N	N	N	N
	47	P	P	N	N	P
	48	N	N	N	N	N
	49	N	N	N	N	N
10	50	N	N	N	N	N
	51	N	P	P	N	P
	52	N	N	N	N	N
	53	N	P	P	N	P
	54	P	P	P	P	N
15	55	N	N	N	N	N
	56	N	N	N	N	N
	57	N	N	N	N	N
	58	N	N	N	N	N
	59	N	N	N	N	N
20	60	N	N	N	N	N
	61	N	N	N	N	N
	62	N	N	N	N	N
	63	N	N	N	N	N
	64	N	N	N	N	N
25	65	N	N	N	N	N
	66	N	N	N	N	N
	67	N	N	N	N	N
	68	N	N	N	N	N
	69	N	N	N	N	N
30	70	P	P	P	P	P
	71	N	N	N	N	N
	72	N	N	N	N	N
	73	P	P	P	P	N
	74	N	N	N	N	N
35	75	N	N	N	N	N
	76	N	N	N	N	P

SUBSTITUTE SHEET

-32-

Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	77	N	N	N	N	N
	78	N	N	N	N	N
	79	N	N	N	N	N
	80	N	N	N	N	N
	81	N	N	N	N	N
10	82	N	N	N	N	N
	83	P	P	N	N	N
	84	N	N	P	N	N
	85	N	N	N	N	N
	86	P	P	P	P	N
15	87	N	N	N	N	N
	88	N	N	N	N	N
	89	P	P	P	P	P
	90	P	P	P	P	N
	91	N	N	N	N	P
20	92	P	P	P	N	N
	93	N	N	N	N	N
	94	N	N	N	N	N
	95	N	N	N	N	N
	96	N	N	N	N	N
25	97	N	N	N	N	N
	98	N	P	P	P	P
	99	P	P	P	P	P
	100	N	N	N	N	N
	101	P	P	P	P	P
30	102	N	N	N	N	N
	103	N	N	N	N	N
	104		N	N	N	N
	105	P	P	P	P	N
	106	N	N	N	N	N
35	107	N	N	N	N	N
	108	N	N	N	N	N
	109	P	P	P	P	P
	110	P	P	P	N	P

SUBSTITUTE SHEET

-33-

		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	111	P	P	P	N	P
	112	N	N	N	N	N
5	113	P	P	P	P	P
	114	N	N	N	N	N
	115	N	N	N	N	N
	116	P	P	P	P	P
	117	N	N	N	N	N
10	118	N	N	N	N	N
	119	N	N	N	N	N
	120	P	P	P	P	P
	121	N	N	N	N	N
	122	N	P	P	N	P
15	123	N	N	N	N	N
	124	N	N	N	N	N
	125	N	N	N	N	N
	126	P	N	N	N	N
	127	N	N	N	N	N
20	128	N	N	N	N	N
	129	N	N	N	N	N
	130	P	P	P	P	N
	131	N	N	N	N	P
	132	N	N	N	N	N
25	133	N	N	N	N	N
	134	N	N	N	N	N
	135	N	N	N	N	N
	136	N	N	N	N	N
	137	N	N	N	N	N
30	138	N	N	N	N	N
	139	N	N	N	N	N
	140	P	N	N	N	N
	141	P	N	P	P	P
	142	N	N	N	N	N
35	143	N	N	N	N	N
	144	N	N	N	N	N

SUBSTITUTE SHEET

-34-

		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	145	N	N	N	N	N
	146	N	N	N	N	N
5	147	N	N	N	N	N
	148	N	N	N	N	N
	149	N	N	N	N	N
	150	N	N	N	N	N
	151	N	N	N	N	N
10	152	N	N	N	N	N
	153	N	N	N	N	N
	154	P	P	P	P	P
	155	N	N	N	N	N
	156	N	N	N	N	N
15	157	N	N	N	N	N
	158	N	N	N	N	N
	159	N	N	N	N	N
	160	N	N	N	N	N
	161	P	P	P	P	P
20	162	N	N	N	N	N
	163	N	N	N	N	N
	164	P	P	P	N	P
	165	N	N	N	N	N
	166	P	P	P	N	P
25	167	N	N	N	N	N
	168	N	N	N	N	N
	169	N	N	N	N	N
	170	N	N	N	N	N
	171	N	N	N	N	N
30	172	N	N	N	N	N
	173	N	N	N	N	N
	174	N	N	N	N	N
	175	N	N	N	N	N
	176	N	N	N	N	N
35	177	N	N	N	N	P
	178	N	N	N	N	N

SUBSTITUTE SHEET

-35-

		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	179	N	N	N	N	N
	180	N	N	N	N	N
5	181	N	N	N	N	N
	182	N	N	N	N	N
	183	P	P	P	P	P
	184	N	N	N	N	N
	185	N	N	N	N	N
10	186	N	N	N	N	N
	187	N	N	N	N	N
	188	N	P	P	N	N
	189	N	N	N	N	N
	190	N	N	N	N	N
15	191	N	N	N	N	N
	192	N	N	N	N	N
	193	N	N	N	N	N
	194	N	N	N	N	N
	195	N	N	N	N	N
20	196	N	N	N	N	N
	197	N	N	N	N	P
	198	P	P	P	N	N
	199	N	N	N	N	P
	200	P	P	P	P	N
25						

The results on the paid donors generally confirms the results from the sera of infected individuals.

30 Example 7: ELISA Determinations of HCV Antibodies Using Combination of HCV Antigens

Plates coated with a combination of C22 and C33c antigens are prepared as follows. A solution containing coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA (25 micrograms/ml), C22 and C33c (2.50 micrograms/ml each) is prepared just prior to addition to the Removeawell

35

**SUBSTITUTE SHEET**

-36-

Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution is added to the plates, they are covered and incubated for 2 hours at 37°C, after which the solution is removed by aspiration.

- 5 The wells are washed once with 400 microliters wash buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100, 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium  
10 phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethylsulfonylfluoride (PMSF)) is added, the plates are loosely covered to prevent evaporation, and are allowed to stand at room temperature for 30 minutes. The wells are then aspirated  
15 to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches with dessicant (3 g Sorb-it™ packs).

- In order to perform the ELISA determination, 20  
20 microliters of serum sample or control sample is added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100, 100 micrograms/ml yeast extract). The  
25 plates are sealed, and are incubated at 37°C for two hours, after which the solution is removed by aspiration, and the wells are washed three times with 400 microliters of wash buffer (phosphate buffered saline (PBS) containing 0.05% Tween 20). The washed wells are treated with 200  
30 microliters of mouse anti-human IgG-horse radish peroxidase (HRP) conjugate contained in a solution of Ortho conjugate diluent (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50% (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM K<sub>3</sub>Fe(CN)<sub>6</sub>, 0.05% (W/  
35 V) Tween 20, 0.02% (W/V) Thimerosal). Treatment is for 1 hour at 37°C, the solution is removed by aspiration, and

SUBSTITUTE SHEET

-37-

the wells are washed three times with 400 ml wash buffer, which is also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) is added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H<sub>2</sub>O<sub>2</sub>. The plates containing the substrate solution are incubated in the dark for 30 minutes at room temperature, the reactions are stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

In a similar manner, ELISAs using fusion proteins of C22 and C33c, and C22, C33c, and S2 and combinations of C22 and C100, C22 and S2, C22 and an NS5 antigen, C22, C33c, and S2, and C22, C100, and S2 may be carried out.

Modifications of the above-described modes for carrying out the invention that are obvious to those of skill in the fields of molecular biology, immunology, and related fields are intended to be within the scope of the following claims.



-38-

Claims

We claim:

- 5           1. A combination of synthetic hepatitis C viral  
(HCV) antigens comprising:  
          (a) a first HCV antigen from the C domain; and  
          (b) at least one additional HCV antigen  
selected from the group consisting of  
10           (i) an HCV antigen from the NS3 domain;  
             (ii) an HCV antigen from the NS4 domain;  
             (iii) an HCV antigen from the S domain;  
and  
             (iv) an HCV antigen from the NS5 domain.  
15
2. A combination of synthetic hepatitis C viral  
(HCV) antigens comprising:  
          (a) a first HCV antigen consisting essentially  
of the C domain; and  
20           (b) a second HCV antigen from the NS3 domain.
3. The combination of claim 2 wherein the first  
HCV antigen is C22 and the second HCV antigen is C33c.
- 25           4. The combination of claim 2 including  
          (c) a third HCV antigen from the S domain.
5. The combination of claim 3 including  
          (c) HCV antigen S2.  
30
6. A combination of synthetic HCV antigens  
comprising:  
          (a) a first HCV antigen consisting essentially  
of the C domain; and  
35           (b) a second HCV antigen from the NS4 domain.

-39-

7. The combination of claim 6 wherein the first HCV antigen is C22 and the second HCV antigen is C100.

5 8. The combination of claim 6 including  
(c) a third HCV antigen from the S domain.

9. The combination of claim 7 including  
(c) HCV antigen S2.

10 10. The combination of claim 1, 2, 3, 4, 5, 6,  
7, 8 or 9 wherein the combination is in the form of a  
fusion polypeptide.

15 11. The combination of claim 1, 2, 3, 4, 5, 6,  
7, 8 or 9 wherein the combination is in the form of said  
first HCV antigen and said additional antigens  
individually bound to a common solid matrix.

20 12. The combination of claim 11 wherein the  
solid matrix is the surface of a microtiter plate well, a  
bead or a dipstick.

25 13. The combination of claim 1, 2, 3, 4, 5, 6,  
7, 8 or 9 wherein the combination is in the form of a  
mixture of said first HCV antigen and said additional HCV  
antigen(s).

30 14. A method for detecting antibodies to  
hepatitis C virus (HCV) in a mammalian body component  
suspected of containing said antibodies comprising  
contacting said body component with the combination of  
synthetic HCV antigens of claim 1, 2, 3, 4, 5, 6, 7, 8, 9,  
10, 11, 12, or 13 under conditions that permit antibody-  
antigen reaction and detecting the presence of immune  
35 complexes of said antibodies and said antigens.

**SUBSTITUTE SHEET**

-40-

15. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with a panel of synthetic HCV antigens comprising:
- (a) a first HCV antigen from the C domain; and
  - (b) at least one additional HCV antigen selected from the group consisting of
    - (i) an HCV antigen from the NS3 domain;
    - (ii) an HCV antigen from the NS4 domain;
    - (iii) an HCV antigen from the S domain;
- and
- (iv) an HCV antigen from the NS5 domain
- under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

16. A kit for carrying out an assay for detecting antibodies to hepatitis C antigen (HCV) in a mammalian body component suspected of containing said antibodies comprising in packaged combination:
- (a) the combination of synthetic HCV antigens of claim 1;
  - (b) standard control reagents; and
  - (c) instructions for carrying out the assay.

SUBSTITUTE SHEET

1 / 26

FIG. 1A

-341 GCCAGCCCCCTGATGGGGCGA  
CGGTGGGGGACTACCCCGCT

-319 CACTCCACCATGAATCACTCCCTGTGAGGAATACTGTCTTCACGCAGAAAGCGTCTAG  
GTGAGGTGGTACTTAGTGAGGGGACACTCTTGATGACAGAAAGTGGCTCTTTCGCAGATC

-259 CCATGGCGTTAGTATGAGTGTGTCGAGCCCTCCAGGACCCCCCTCCCGGGAGAGCCATA  
GGTACCGCAATCATACTCACAGCACGTCGAGGTCTCTGGGGGAGGGCCCTCTCGGTAT

-199 GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACGGGTCTTCTTGGA  
CACCAGAGCCCTTGGCCACTCATGTGGCCTTAACGGTCTCTGCTGGCCCCAGGAAAGAACCT

-139 TCAACCCGCTCAATGCCCTGGAGATTGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGT  
AGTTGGCGGAGTTACGGACCTCTAAACCCGACGGGGCGTTCTGACGATCGGCTCATCA

- 79 GTTGGGTGCGGAAGGCCCTTGTGGTACTGCCCTGATAGGTGCTTGCGAGTGCCCCGGGAG  
CAACCCAGCGCTTTCGGGAACACCATGACGGACTATCCACGAAACGCTACGGGGCCCTC

- 19 GTCTCGTAGACCGTGCAAC  
CAGAGCATCTGGCACGTGG

Arg Thr

MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln  
1 ATGAGCACGAATCCTAAACCTCAAAAAAACAACCAACCGTAACACCAACCGTCGCCACAG  
TACTCGTGCTTAGGATTGGAGTTTTTTGTGTTGCAATTGTGGTTGGCAGCGGGTGTC

AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg  
61 GACGTCAAGTTCCCGGGTGGCGGTGAGTCTGTTGGAGTTTACTTGTGTCGCCGCGCAGG  
CTGCAGTTCAAGGGCCCCACCGCCAGTCTAGCAACCAACCTCAAAATGAACAACGGGGCGGTCC

SUBSTITUTE SHEET

2 / 26

121 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly  
GGCCCTAGATTGGGTGTGCGCGACGAGAAAGACTTCCGAGCGGTCCGCAACCTCGAGGT  
CCGGGATCTAACCACACACGCGCGTCTCTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA

181 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly  
AGACGTCAGCCTATCCCCAAGGCTCGTCGCCCCAGGGCAGGACCTGGGCTCAGCCCCGG  
TCTGCAGTCGGATAGGGTTCCGAGCAGCCGGGCTCCCGTCTTGACCCGAGTCGGGCCC

241 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro  
TACCCCTGGCCCCCTCTATGGCAATGAGGGCTGCGGGTGGCGGATGGCTCCTGTCTCCC  
ATGGGAACCGGGAGATACCGTTACTCCGACGCCCCACCGCCCTACCGAGGACAGAGG

301 ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly  
CGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCCGGCGTAGGTCCGCCAATTGGGT  
GCACCGAGAGCCGGATCGACCCCGGGTGTCTGGGGCGCCCATCCAGCGCGTTAAACCCA

361 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal  
AAGGTCATCGATACCCCTACGTGGGCTTCGCCGACCTCATGGGTACATACCGCTCGTC  
TTCCAGTAGCTATGGGAATGCACGCCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG

421 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp  
GGCGCCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGAC  
CCCGGGGGAGAACCTCCCGGACGGTCCCGGGACCGCGTACCGCAGGCCCAAGACCTTCTG

FIG. 1B

3 / 26

FIG. 1C

Thr

481 GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla  
GGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTCTCTATCTTCTGCGC  
CCGCACTTGATACGTTGTCCTTGGAAGGACCAACGAGAAAGATAGAAAGAACCCGG

541 LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu  
CTGCTCTCTTGCTTGACTGTGCCGCTTCGGCCTACCAAGTGCGCAACTCCACGGGCTT  
GACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTACGCCGTTGAGGTGCCCCCGAA

601 TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle  
TACCACGTCACCAATGATGTCCTTAACCTCGAGTATTGTGTACGAGCGCGCGATGCCATC  
ATGGTGCACTGGTTACTAACGGGATTGAGCTCATACACATGCTCCGCCGGCTACGGTAG

661 LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal  
CTGCACACTCCGGGTGCGTCCCTTGCGTTCGTGAGGGCAACGCCCTCGAGGTGTGGGTG  
GACGTGTGAGGCCCCACGACGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACAAACCCAC

721 AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg  
GCGATGACCCCTACGGTGGCCACCAAGGATGGCAAACTCCCCCGACGACGCTTCGACGT  
CGCTACTGGGGATGCCACCGGTGTCCTACCGTTTGAGGGGCGCTGCGTCGAAGCTGCA

781 HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu  
CACATCGATCTGCTTGTCGGGAGCGCCACCTCTGTTTCGGCCCTCTACGTGGGGACCTA  
GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGACACCCCTGGAT

841 CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr  
TGCGGGTCTGTCTTTCTTGTCGGGCCAACTGTTACCTTCTCTCCAGCGCCACTGGACG  
ACGCCACAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAGAGGGTCCCGGTGACCTGC

4 / 26

ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp  
 ACGCAAGGTTGCAATTGCTCTATCTATCCCGCCATATAACGGGTCAACGCATGGCATGG  
 TCGGTTCCAAACGTTAACGAGATAGATAGGCGCGGTATATTGCCAGTGGGTACCGTACC  
 901

AspMetMetMetAsnTrpSerProThrAlaLeuValMetAlaGlnLeuLeuArgIle  
 GATATGATGATGAACCTGGTCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATC  
 CTATACTACTACTTGACCAAGGGGATGCTGCCGCAACCATTAACCGAGTCGACGAGGCCCTAG  
 961

Val  
 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla  
 CCACAAGCCATCTTGGACATGATCGCTGGTCTCACTGGGAGTCCTGGCGGCATAGCG  
 GGTGTTCCGTAGAACCTGTACTAGCGACCAAGTGAACCCCTCAGGACCGCCCCGTATCGC  
 1021

TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuPheAlaGly  
 TATTTCCTCCATGGTGGGAACCTGGCGAAGGTCTCTGGTAGTGTCTGTCTATTTGCCCGC  
 ATAAAGAGGTACCAACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCG  
 1081

ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal  
 GTCGACGCGGAACCCACGTCACCGGGGAAGTCCCGGCCACACTGTGTCTGGATTGTGT  
 CAGCTGCCGCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGTGTACACAGACCTAAACAA  
 1141

SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp  
 AGCCTCCTCGCACCGCGCCCAAGCAGAACGTCCAGCTGATCAACACCAACGGCAGTTGG  
 TCGGAGGAGCGTGGTCCCGGTTCTGTCAGGTTCGACTAGTTGTGGTTGCCGTCAACC  
 1201

FIG. 1D

5 / 26

## FIG. 1E

1261 HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly  
 CACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAACACCGGCTGGTTGGCAGGG  
 GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC  
  
 1321 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg  
 CTTTTCATACACCACAAGTTCAACTCTTCAGGCTGTCTGAGAGGCTAGCCAGCTGCCGA  
 GAAAGATAGTGGTGTTCAGTTGAGAACTCCGACAGGACTCTCCGATCGGTCGACGGCT  
  
 1381 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro  
 CCCCTTACCGATTTTGACACGAGGCTGGGCCCTATCAGTTATGCCAACGGAGCGCCCC  
 GGGGAATGGCTAAACTGGTCCCGACCCCGGATAGTCAATACGGTTGCCCTTCGCCCGGG  
  
 1441 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys  
 GACCAGCGCCCTACTGCTGGCACTACCCCAAAACCTTGCGGTATTGTGCCCGCGAAG  
 CTGGTCGCGGGGATGACGACCGTGATGGGGGTTTGGAACGCCCATAAACACGGGCGCTTC  
  
 1501 SerValCysGlyProValTyrCysPheThrProSerProValValGlyThrThrAsp  
 AGTGTGTGTGGTCCGGTATATTGCTTCACTCCAGCCCGGTGGTGGGAAACGACCGAC  
 TCACACACACAGGCCATATAACGAAGTGAGGGTCGGGGCACCAACCCCTTGCTGGCTG  
  
 1561 ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn  
 AGGTCGGGGCGGCCACCTACAGCTGGGTGAAATGATACGGACGCTCTTCGTCCTTAAC  
 TCCAGCCCCGCGGGTGATGTGACCCCACTTTTACTATGCCTGCAGAACGAGGAATTG  
  
 1621 AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe  
 AATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTGGATGAACCTCAACTGGATT  
 TTATGTCGCGGTGGCGACCCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG



6 / 26

1681 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis  
 ACCAAAGTGTGGAGCGCCTCCTTGTGTATCGGAGGGCGGCAACAACACCTGCAC  
 TGGTTTCACACGCCCTCGCGGAGGAACACAGTAGCCTCCCCGCCCTGTGTGGACGTG

1741 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly  
 TGCCCCACTGATGTCTCCGCAAGCATCCGGACGCCACATACCTCTCGGTGCGCTCCGGT  
 ACGGGGTGACTAACGAAGCGCTTCGTAGGCCTGCGGTGTATGAGAGCCACGCCGAGGCCA

## Ile

1801 ProTrpLeuThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys  
 CCTGGATCACACCCAGGTGCTGGTCCGACTACCCGTATAGGCTTTGGCATTATCCTTGT  
 GGGACCTAGTGTGGTCCACGGACCGACTGATGGGCATATCCGAAACCGTAATAGGAACA

1861 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu  
 ACCATCAACTACACCATATTTAAATCAGGATGTACGTGGGAGGGTCCGAACACAGGCTG  
 TGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCTCCCCAGCTTGTGTCCGAC

1921 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer  
 GAAGCTGCCCTGCAACTGGACCGGGGCGGAACGTTGCGATCTGGAAGACAGGACAGGTCC  
 CTTCGACGGACGTTGACCTGCGCCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGG

1981 GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr  
 GAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTCTCCCGTGTCTCTTCACA  
 CTCGAGTCGGGCAATGACGACTGGTGTGTGTACCCGTCCAGGAGGGCACAAAGGAAGTGT

FIG. 1F

7 / 26

## FIG. 1G

2041 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln  
 ACCCTACCAGCCTTGTCACCGCCTCATCCACCTCCACAGAACATTTGGACGTGCAG  
 TGGGATGGTCGGAACAGGTGGCCGAGTAGGTGGAGGTGGTCTTGTAAACACCTGCACGTC  
  
 2101 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal  
 TACTTGTAAGGGTGGGTCAAGCATCGCTCCTGGGCCATTAAAGTGGAGTACGTCTGTT  
 ATGAACATGCCCCACCCAGTTCTGTAGCGCAGGACCCGGTAATTCAACCTCATGCAGCAA  
  
 2161 LeuLeuPheLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu  
 CTCCTGTTCTCTGCTTGACAGACGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTC  
 GAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAG  
  
 2221 IleSerGlnAlaGluAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla  
 ATATCCCAAGCGGAGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCC  
 TATAGGTTTCGCCCTCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCGTAGGGACCCGG  
  
 2281 GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly  
 GGGACGCACGGTCTTGATCCTTCCTCGTGTCTTCTGCTTTGCATGGTATTTGAAGGGT  
 CCCTGCGTGCCAGAACATAGGAAGGAGCACAAAGAACGAAACGTACCATAACTTCCCCA  
  
 2341 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeu  
 AAGTGGGTGCCCGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTGCTCCTG  
 TTCACCCACGGGCTCGCCAGATGTGGAAGATGCCCTACACCGGAGGAGGACGAGGAC  
  
 2401 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly  
 TTGGCGTTGCCCCAGCGGCGTACGCGCTGGACACGAGGTGGCCCGCTCGTGTGGCGGT  
 AACCGCAACGGGGTCCGCCCATGCGCGACCTGTGCTCCACCGGCGCAGCACACCGCCA

8 / 26

2461 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer  
 GTTGTTCGTCGGGTGATGGCGCTGACTCTGTCCACCATATTACAAGCGTATATCAGC  
 CAACAAGAGCAGCCCACTACCGCGACTGAGACAGTGGTATAATGTTCCGGATATAGTCG  
  
 (Asn)  
 2521 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp  
 TGGTGCTTGTGGCTTCAGTATTTCTGACCAAGAGTGGAAAGCGCAACTGCACGTGTGG  
 ACCACGAACACCAACCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTGCACACC  
  
 IleProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal  
 2581 ATTCCCCCCTCAACGTCGAGGGGGCGCGACGCGTCATCTTACTCATGTGTGCTGTA  
 TAAGGGGGAGTTGCAGGCTCCCCCGGCTGCCGAGTAGAATGAGTACACACGACAT  
  
 HisProThrLeuValPheAspIleThrLysLeuLeuAlaValPheGlyProLeuTrp  
 2641 CACCCGACTCTGGTATTGACATCACCAAAATTGCTGCTGGCGTCTTCGGACCCCTTTGG  
 GTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGACAGCCTGGGAAACC  
  
 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg  
 2701 ATTCTTCAAGCCAGTTTGCTTAAAGTACCCCTACTTTGTGCGCGTCCAAGGCCCTTCTCCGG  
 TAAGAAGTTCGGTCAACCGAATTTTCATGGGATGAAACACCGCGCAGGTTCGGGAAGAGGCC  
  
 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys  
 2761 TTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAAATGGTCATCATTAAG  
 AAGACGGCAATCGCGCCTTCTACTAGCCTCCGGTAATGACGTTTACCAGTAGTAATTC

FIG. 1H

## FIG. 11

2821 LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla  
TTAGGGCGCCTTACTGGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGACTGGGCG  
AATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAACCCCTGACCCCGC  
2881 HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu  
CACAAACGGCTTGCGAGATCTGGCCGCTGGCTGTAGAGCCAGTCGTCTTCTCCCAAATGGAG  
GTGTTGCCGAACGCTCTAGACCGGACCGACATCTCGGTACGAGAGAGGGTTTACCTC  
2941 ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu  
ACCAAGCTCATCACGTGGGGGCGAGATACCGCCGCTGCGGTGACATCATCAACGGCTTG  
TGGTTCGAGTAGTGACACCCCGCTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAAC  
3001 ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer  
CCTGTTTCCCGCCGAGGGCGCGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC  
GGACAAAGGCGGCTCCCGGCCCTCTATGACGAGCCCGGTGCGCTACCTTACCAGAGG  
3061 LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu  
AAGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCCAGCAGACAAAGGGCCTCCTA  
TTCCCCACCTCCAAACGACCGCGGTAGTGCCCGCATGCGGGTCTGTCTTCCCCGGAGGAT  
3121 GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln  
GGGTGCATAATCACACGCTAACTGGCCGGGACAAACCAAGTGAGGGTGAGGTCCAG  
CCCACGTATTAGTGGTGGATTGACCGGCCCTGTTTTGTGTTACCTCCCACTCCAGGTC  
3181 IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr  
ATTGTGTCAACTGCTGCCCAACCTTCTGGCAACGTGCATCAATGGGGTGTGTGGACT  
TAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCAACGACCTGA

10 / 26

3241	ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet GTCTACCACGGGCGGAAACGAGGACCATCGCGTCACCCAAAGGTCTCTCATCCAGATG CAGATGGTGCCCCCGCCTTGCTCCTGTAGCGCAGTGGGTCCAGGACAGTAGGTCTAC
3301	TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu TATACCAATGTAGACCAAGACCTTGTTGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTTG ATATGGTTACATCTGTTCTGGAAACACCCGACCGGGCGAGCGTTCCATCGGCGGAGTAAC
3361	ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle ACACCCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATT TGTGGACGTGAACGCCGAGAGCCTGGAAATGGACCATGCTCCGTGCGGCTACAGTAA
3421	ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr CCCGTGCGCGCGGGGTGATAGCAGGGCAGCCTGTCTGTCGCCCGGCCCATTTCTCTAC GGCACGCGCGCGCCCACTATCGTCCCGTCGACGACAGCGGGCGGGTAAAGGATG
3481	LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe TTGAAAGGCTCCTCGGGGGTCCGCTGTGTGTCGCCCGGGGCACGCCGTGGCATATTT AACTTCCGAGGAGCCCCCAGGCGACAACACGGGGCCCCCGTGGCGCACCCGTATAAA
3541	ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn AGGCCCGGGTGTGCACCCGTGGAGTGGCTAAGCGGTGGACTTTATCCCTGTGGAGAAC TCCCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG

FIG. 1J

11 / 26

## FIG. 1K

3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro  
 CTAGAGACAACCATGAGGTCCCGGTGTTACGGATAACTCCTCTCCACCAGTAGTGCCC  
 GATCTGTGTGTA CTCCAGGGGCCACAAGTGCCCTATTGAGGAGAGGTGGTCATCACGGG  
  
 3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal  
 CAGAGCTTCCAGGTGGCTACCTCCATGTCTCCACAGGCAGCGCAAAAGCACCAAGGTC  
 GTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCCCGCTTTTCGTGTTCCAG  
  
 3721 ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla  
 CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTGCTGCA  
 GGCCGACGTATACGTCGAGTCCCGATATTCACGATCATGAGTTGGGGAGACAAACGCGT  
  
 3781 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr  
 ACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACC  
 TGTGACCCCGAAACCAACGAATGTACAGGTTCCGAGTACCCCTAGTAGGATTGTAGTCCTGG  
  
 3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu  
 GGGTGAGAAACAATTACCCTGGCAGCCCCCATCAGTACTCCACCTACGGCAAGTTCCTT  
 CCCCACCTCTTGTTAATGGTGACCCGTCCGGGTAGTGCAATGAGGTGGATGCCGTTCAGGAA  
  
 3901 AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer  
 GCCGACGGCGGTGCTCGGGGGCGGCTTATGACATAATAATTGTGACGAGTGCCACTCC  
 CGGCTGCCGCCACGAGCCCCCGGGAATACTGTATTATTAAACACTGCTCACGGTGAGG

12 / 26

(Val)

3961 ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly  
ACGGATGCCACATCCATCTTGGGCATCGGCATCTCCTTGACCAAGCAGAGACTGCGGGG  
TGCCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACCTGGTTCTCTCTGACGCCCC

4021 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro  
GCGAGACTGTTGTGCTCGCCACCGCCACCCCTCCGGCTCCGTCACTGTGCCCATCCC  
CGCTCTGACCAACACGAGCGGTGGCGTGGGAGGCCCGAGGAGTGACACGCGGTAGGG

4081 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle  
AACATCGAGGAGGTGCTCTGTCCACCAACCGGAGAGATCCCTTTTACGGCAAGGCTATC  
TTGTAGCTCCTCCAACGAGACAGGTGTGGCTCTCTAGGGAAAAATGCCGTTCCGATAG

4141 ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysCys  
CCCCTCGAAGTAATCAAGGGGGGAGACATCTCATCTTCTGTTCATTCAAAGAAAGATGC  
GGGAGCTTCATTAGTTCCCCCTCTGTAGAGTAGAAGACAGTAAGTTCTTCTTCCAGC

4201 AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly  
GACGAACCTCGCCGCAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGT  
CTGCTTGAGCGCGGTTTCGACCCAGCGTAACCCGTAGTTACGGCACCCGGATGATGGCGCCA

4261 LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu  
CTTGACGTGTCCGTCAATCCCGACCGACCGCGATGTTGTCTGTCGTGGCAACCGATGCCCTC  
GAACTGCACAGGCAGTAGGGCTGGTCGCCCTACAAACAGCAGCACCCGTTGGCTACGGGAG

4321 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln  
Tyr  
ATGACCGGCTATACCGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTACCCAG  
TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC

FIG. 1L

13 / 26

## FIG. 1M

(Ser)

ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp  
 4381 ACAGTCGATTTCAGCCTTGACCCCTACCTTCACCATTGAGACAATCACGCTCCCCCAGGAT  
 TGTCAAGCTAAAGTCGGAAGTGGATGGAAGTGGTAACTCTGTAGTGCAGGGGGTCCCTA  
  
 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg  
 4441 GCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGAAGCCAGGCATCTACAGA  
 CGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCCTTCGGTCCGTAGATGTCT  
  
 PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys  
 4501 TTTGTGGCACCGGGAGCGCCCCCTCCGGCATGTTTCGACTCGTCCGTCTCTGTGAGTGC  
 AAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCAGGACACTCACCG  
  
 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg  
 4561 TATGACGCAGGCTGTGCTTGATAGCTACGCCCGCCGAGACTACAGTTAGGCTACGA  
 ATACTGCGTCCGACACGAACCATACTCGAGTGGGGGGCTCTGATGTCAATCCGATGCT  
  
 AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly  
 4621 GCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCATCTTGAAATTTTGGGAGGGC  
 CGCATGTACTTGTGGGGCCCCGAAAGGGCACACGGTCCCTGCTAGAACTTAAACCCCTCCCG  
  
 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly  
 4681 GTCTTTACAGGCCCTCACTCATATAGATGCCCACTTCTATCCACAGACAAGCAGAGTGGG  
 CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCTCTCACCC  
  
 GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro  
 4741 GAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCCT  
 CTCTTGGAAGGAATGGACCATCGCATGTTCCGTGGCACACCGCATCCCGAGTTCGGGGA



14 / 26

4801 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly  
CCCCATCGTGGACACAGATGTGGAAGTGTGATTGCGCTCAAGCCACCCCTCCATGGG  
GGGGTAGCACCCCTGGTCTACACCTTCACAACTAAGCGGAGTTCGGGTGGAGGTACCC

4861 ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro  
CCAAACACCCCTGCTATACAGACTGGGCGCTGTTTCAGAAATCAACCTGACGCACCCA  
GGTTGTGGGACGATATGTCTGACCCCGCACAAAGTCTTACTTTAGTGGGACTGCCGTGGGT

4921 ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp  
GTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGG  
CAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACC

4981 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal  
GTGCTCGTTGGCGGCTCCTGGCTGCTTTGGCCGCGTATTGCCGTCAACAGGCTGCGTG  
CACGAGCAACCGCCGACGACCGAAACCGGCGCATACGGACAGTTGTCCGACGCAC

5041 ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal  
GTCATAGTGGCAGGTCGTCCTTGTCGGGAAGCCGGCAATCATACCTGACAGGGAAGTC  
CAGTATCACCCGTCGCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAG

5101 LeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln  
CTCTACCGAGAGTTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAA  
GAGATGGCTCTCAAGCTACTCTACCTTCTCAGCAGAGTCGTGAATGGCATGTAGCTCGTT

FIG. 1N

15/ 26

## FIG. 10

GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer  
 5161 GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCCTCCTGCAGACCCGGTCC  
 CCTACTACGAGCGGCTCGTCAAGTTCTTCCGGGAGCCGGAGACGTCTGGCGCAGG  
  
 ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe  
 5221 CGTCAGGCAGAGGTTATCGCCCTGCTGTCCAGACCAACTGGCAAAACTCGAGACCTTC  
 GCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAG  
  
 TrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr  
 5281 TGGCGGAAGCATATGTGGAACCTTCATCAGTGGGATACAATACTTGGCGGGCTTGTCAACG  
 ACCCGCTTCGTATACACCTTGAAGTAGTCACCCCTATGTTATGAACCGCCCGAACAGTTGC  
  
 LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro  
 5341 CTGCCTGGTAACCCCGCATTTGCTTCATTGATGGCTTTTACAGCTGCTGTACCAAGCCCA  
 GACGGACCATTGGGGCGGTAAACGAAGTAACTACCGAAATGTCGACGACAGTGGTCCGGT  
  
 LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu  
 5401 CTAACCACTAGCCAAACCCCTCCTCTTCAACATATTGGGGGGGTGGTGGCTGCCCCAGCTC  
 GATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCAACCCACCGGGTCCGAG  
  
 AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly  
 5461 GCCGCCCGGTGCCGCTACTGCCCTTTGTGGCGGCTGGCTTAGCTGGCGCCGCATCGGC  
 CGGCGGGGCCACGGCGATGACGGAAACACCCCGGACCCGAATCGACCGCGGGGTAGCCG  
  
 SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla  
 5521 AGTGTTGGAAGGAGGTCCTCATAGACATCCTTGCAGGGTATGGCGCGGGCGTGGCG  
 TCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCCG

16 / 26

(Gly)

5581 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal  
 GGAGCTCTTGTCGCAATCAAGATCATGAGCGGTGAGGTCCCTCCACGGAGGACCTGGTC  
 CCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCCAG

5641 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla  
 AATCTACTGCCCGCATCCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCA  
 TTAGATGACGGCGGTAGGAGAGCGGCCCTCGGGAGCATCAGCCGCACCCAGACACGTCGT

5701 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle  
 ATACTGCCCGCCGACGTTGCCCGGCGAGGGGCAGTGCAGTGGATGAACCGGCTGATA  
 TATGACGGCGCGGTGCAACCGGGCCGCTCCCCCGTCACGTCACTTGGCCGACTAT

5761 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla  
 GCCTTCGCCCTCCCGGGGAACCATGTTTCCCCCACGCACTACGTGCCGGAGAGCGATGCA  
 CGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGTGCGTGATGCACGGCCCTCTCGCTACGT

(HisCys)

5821 AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu  
 GCTGCCCGGTCACTGCCATACTCAGCAGCCCTCACTGTAAACCCAGCTCCTGAGCGGACTG  
 CGACGGCGCAGTGACGGTATGAGTCGTCCGGAGTGACATTGGGTCCAGGACTCCCGCTGAC

5881 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle  
 CACCAAGTGGATAAGCTCGGAGTGTAACCACTCCATGCTCCGGTTCTCGGCTAAGGGACATC  
 GTGGTCACCTATTTCGAGCCCTCACATGTTGAGGTACGAGGCCCAAGGACCGATTCCCTGTAG

FIG. 1P

17 / 26

## FIG. 1Q

5941 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet  
 TGGGACTGGATATCGAGGTGTGAGCGACTTAAAGACCTGGCTAAAGCTAAGCTCATG  
 ACCCTGACCTATACGCTCCACAACACTCGCTGAAATTTCTGGACCGATTTCGATTCCGAGTAC  
  
 6001 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg  
 CCACAGCTGCCTGGATCCCCTTTGTCTCCTGCCAGCGGGGTATAAGGGGTCTGGCGGA  
 GGTGTCCAGCGACCCCTAGGGGAAACACAGGACGGTCGCGCCCATATTTCCCCAGACCGCT  
 (Val)  
 6061 GlyAspGlyIleMethIleThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys  
 GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAA  
 CACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT  
  
 6121 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe  
 AACGGGACGATGAGGATCGTCGGTCTCTAGGACCTGCAGGAACAATGTGGAGTGGACCTTC  
 TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTGTAACCTCACCTCACCTGGAAG  
  
 6181 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe  
 CCCATTAAATGCCCTACACCGGGCCCTGTACCCCCCTTCCTGCGCCGAACACTACACGTTTC  
 GGGTAATTACGGATGTGTGTCGCCGGGACATGGGGGAGGACGCGGCTTGATGTGCAAG  
  
 6241 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis  
 GCGCTATGGAGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGACTTCCAC  
 CGCGATACCTCCCAACAGACGTCTCCTTATACACCTCTATTTCCGTCCACCCCCCTGAAGTG  
  
 6301 TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu  
 TAGGTACGGGTATGACTACTGACAAATCTCAAAATGCCCGTCCAGGTCCCATCGCCCCGAA  
 ATGCACTGCCCCATACTGATGACTGTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTT

18 / 26

6361 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu  
TTT'TT'CACAGAA'TTGGACGGGTGCGCTACATAGTTTGGCCCCCCTGCAAGCCCTTG  
AAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGCGGGGACGTTCCGGGAAC

6421 LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu  
CTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGGTCGCAATTA  
GACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATGGCCATCCCAGCGTTAAT

6481 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis  
CCTTGCGAGCCCCGAACCGGACGTGGCCGTGTGACGTCCATGTCTCACTGATCCCCTCCCAT  
GGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGTACTAGGGAGGGTA

6541 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer  
ATAACAGCAGAGCGCGCGGCGGAAGTTGGCGAGGGGATCACCCCTCTGTGGCCAGC  
TATTGTCTCTCCGCGCGCCCTTCCAAACCGCTCCCCCTAGTGGGGGAGACACCGGTCG

6601 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp  
TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCCGCTAACCATGAC  
AGGAGCCGATCGGTCGATAGCGGAGGTAGAGATTCCGTTGAACGTGGCGATTGGTACTG

6661 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn  
TCCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGCGGCAAC  
AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCCCTCTACCCCGCGTTG

FIG. 1R

19 / 26

## FIG. 1S

IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal  
 6721 ATCAACAGGGTTGAGTCAGAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTGTG  
 TAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAAGACCTGAGGAAGCTAGGCCGACAC  
  
 AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg  
 6781 GCGAGGAGGACGAGCGGAGATCTCCGTACCCGCAAAATCCTGCCGAAAGTCTCGGAGA  
 CGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGCGCTCTTAGGACGCCCTCAGAGCCTCT  
  
 PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr  
 6841 TTCGCCACAGGCCCTGCCCGTTTGGCGCGCGGCACTATAACCCCGCTAGTGGAGACG  
 AAGCGGTCCGGACGGGCAACCCCGCGCCTGATATTGGGGGGCGGATCACCTCTGC  
  
 TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProLys  
 6901 TGGAAAAGCCCGACTACGAACCACTGTGTCCATGGCTGTCCGCTTCCACCTCCAAAG  
 ACCTTTTTCGGGCTGATGCTTGGTGGACACCAAGGTACCGACAGCGGAAGTGGAGGTTTC  
  
 SerProProValProProArgLysLysArgThrValValLeuThrGluSerThrLeu  
 6961 TCCCCCTCCTGTGCCCTCCGCCCTCGGAAGAGCGGACGGTGGTCCCTCACTGAATCAACCTA  
 AGGGAGGACACGGAGCGGAGCCTTCTTCGCCCTGCCACCAAGGAGTACTTAGTTGGGAT  
  
 (Ser)  
 SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle  
 7021 TCTACTGCCCTTGGCCGAGCTCGCCACCAAGAGCTTTGGCAGCTCCTCAACTTCCGGCATT  
 AGATGACGGAACCGGCTCGAGCGGTGCTTCGAAACCGTCGAGGAGTTGAAGCCGTAA  
  
 ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer  
 7081 ACGGGCACAATACGACAACATCCTCTGAGCCCGCCCTTCTGGCTGCCCCCGACTCC  
 TGCCCCGCTGTTATGCTGTTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGCTGAGG

20/ 26

(PheAla)

7141 AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu  
 GACGCTGAGTCCTATTCTCCATGCCCCCTGGAGGGGAGCCTGGGATCCGGATCTT  
 CTGCGACTCAGGATAAGGAGGTACGGGGGGACCTCCCCCTCGGACCCCTAGGCCCTAGAA

7201 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys  
 AGCGACGGGTCAATGTCAGTCAAGGTGAGGCCAACCGCGGAGGATGTCGTGTGCTGC  
 TCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGCCTCCTACGACACGACG

7261 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys  
 TCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGC CGCGGAAGAACAGAAA  
 AGTTACAGAAATGAGAACCTGTCCCGGTGAGCAGTGGGGCACGCGCGCCTTCTGTCTTT

7321 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisAsnLeuValTyrSerThr  
 CTGCCCCATCAATGCACCTAAGCAACTCGTTGCTACGTCAACCAATTTGGTGTATCCACC  
 GACGGGTAGTTACGTGATTTCGTTGAGCAACGATGCAGTGTGTTAAACCATATAAGGTGG

7381 ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu  
 ACCTCACGCAGTGCTTGCCAAAGGCAGAGAAAGTCACATTTGACACAGACTGCAAGTCTG  
 TGGAGTGGTCAACGAAACGGTTTCCGTCTTCTTTCAGTGTAAACTGTCTGACGTTCAAGAC

7441 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaSerLysValLysAla  
 GACAGCCATTACGAGGACGTACTCAAGGAGGTTAAAGCAGCGCGGTCAAAAGTGAAGGCT  
 CTGTCCGGTAATGGTCCCTGCATGAGTTCCTCCAATTTTCGTGCGCCGAGTTTTCACCTTCCGA

FIG. 1T

21 / 26

## FIG. 1U

(Phe)

7501 AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys  
 AACTTGCTATCCGTAGAGGAAGCTTGACGCTGACGCCCCCACACTCAGCCAAATCCAAG  
 TTGAACGATAGGCATCTCCTTCGAACGTCGGACTGCGGGGTGTGAGTCGGTTAGGTTT  
  
 7561 PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn  
 TTTGGTTATGGGGCAAAAGACGTCCTGCTGCCATGCCAGAAAGGCCGTAAACCCACATCAAC  
 AAACCAATACCCCGTTTCTGTCAGGCAACGGTACGGTCTTTCCGGCATTTGGGTGTAGTTG  
  
 7621 SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla  
 TCCGTGTGGAAGACCTTCTGGAAGACAAATGTAAACACCAATAGACACTACCATCATGGCT  
 AGGCACACCTTTCTGGAGACCTTCTGTATACATTGTGGTTATCTGTGATGGTAGTACCGA  
  
 7681 LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle  
 AAGAACGAGGTTTCTGCGTTCAGCCTGAGAAAGGGGGTCTGTAAGCCAGCTCGTCTCATC  
 TTCTTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCAGCATTCGGTCCGAGCAGTAG  
  
 7741 ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr  
 GTGTTCCCCGATCTGGCGTGCGCGTGTCGAAAGATGGCTTTGTACGACCGTGGTTACA  
 CACAAGGGCTAGACCCGCGCACACGCTTTTCTACCGAAACATGCTGCACCAATGT  
  
 7801 LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg  
 AAGTCCCCCTTGCGCGTGATGGGAAGCTCCTACGGATTCCAATACTCACGACAGACGGG  
 TTCGAGGGGAACCGCACTACCCCTTCGAGGATGCCTAAGGTTATGAGTGGTCTGTGCGC  
  
 7861 ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp  
 GTTGAATTCCCTCGTGCAAGCGTGGAAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGAT  
 CAACTTAAGGAGCACGTTCCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA



22 / 26

7921 ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr  
 ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTAC  
 TGGGGCAGCGAAACTGAGGTGTCACTGAGTCACTCTCGTGTAGGCATGCCTCCTCCGTTAGATG

7981 GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu  
 CAATGTTGTGACCTCGACCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTT  
 GTTACAACACTGGAGCTGGGGGTTTCGGGGCCACCGGTAGTTCAGGGAGTGGCTCTCCGAA

8041 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg  
 (Gly)  
 TATGTTGGGGCCCTCTTACCAATTCAAGGGGGAGAACTCGGCTATCGCAGGTGCCGC  
 ATACAACCCCCGGGAGAAATGGTTAAGTTCCCCCTCTTGACGCCGATAGCGTCCACGGCG

8101 AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg  
 GCGAGCGCGTACTGACAACTAGCTGTGTTAACACCCCTCACTTGCTACATCAAGGCCCGG  
 CGCTCGCCGATGACTGTTGATCGACACCATTTGTGGGAGTGAACGATGTAGTTCCGGGCC

8161 AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu  
 GCAGCCTGTCGAGCCGCGAGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA  
 CGTCGGACAGCTCGGCGTCCCGAGGTCTGACGTGGTACGAGCACACACCGCTGCTGAAT

8221 ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr  
 GTCGTTATCTGTGAAGCGGGGGTCCAGGAGGACCGGGCGAGCCTGAGAGCCTTCACG  
 CAGCAATAGACACTTTCGGCGCCCCCAGGTCTCTCGGCCCGCTCGGACTCTCGGAAGTGC

FIG. 1V

23/ 26

## FIG. 1W

8281	GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu GAGGCTATGACCAGGTACTCGCCCTCGGGACCCCCACAAACCAGAAATACGACTTG CTCCGATACTGGTCCATGAGCGGGGGACCCCTGGGGGTGTGGTCTTATGCTGAAC
8341	GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg GAGCTCATAACATCATGCTCCTCCAACGTGTCACTCGCCACGACGGCGCTGGAAAGAGG CTCGAGTATTGTAGTACGAGGAGGTGACAGTCAGCGGTGCTGCCGACCTTCTCC
8401	ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla GTCTACTACCTCACCCGTGACCCCTACAACCCCTCGCGAGAGCTGCGTGGAGACAGCA CAGATGATGGAGTGGCACTGGATGTTGGGGGAGCGCTCTCGACGCCCTCTGTCTCGT
8461	ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp AGACACACTCCAGTCAATTCTTGCTAGGCAACATAATCATGTTTGGCCCCACACTGTGG TCTGTGTGAGTCAAGTAAAGGACCGATCCGTGTATTAGTACAAACGGGGGTGTGACACC
8521	AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu GCGAGGATGATGATGATGACCCATTCTTTAGCGTCCTTATAGCCAGGACCGCTTGAA CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCAACTT
8581	GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro CAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGAACCACTTGATCTACCT GTCCGGGAGCTAACGCTCTAGATGCCCCGACGATGAGGTATCTTGGTGAACATAGATGGA
8641	ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT GGTTAGTAAGTTTCTGAGGTACCGGAGTCGGTAAAGTGAGGTGTCAATGAGAGGTCCA

24 / 26

8701 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp  
GAAATTAATAGGTGGCCGATGCCCTCAGAAAACCTGGGGTACCGCCCTTGCGAGCTTGG  
CTTTAATTATCCACCGCGGTACGGAGTCTTTTGAACCCCATGCGCGGAAACGCTCGAACCC

Gly  
8761 ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle  
AGACACCGGCGCGAGCGTCCGCGTAGGCTTCTGGCCAGAGGAGGAGGCTGCCATA  
TCTGTGGCCCGGCGCTCGCAGGCGGATCCGAAGACCGGTCTCCTCCGTCCCGACGGTAT

8821 CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla  
TGTGGCAAGTACCTCTTCAACTGGGCGAGTAAGAAACAAGCTCAAACCTCACTCCAATAGCG  
ACACCGTTCATGGAGAAAGTTGACCCGTCATTCTTGTTCGAGTTTGAGTGAGGTTATCGC

8881 AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle  
GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGGCTACAGCGGGGAGACATT  
CGCGACCGGTCGACCTGAACAGGCGCGACCAAGTGCCGACCGATGTGCGCCCCCTCTGTAA

8941 TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuAla  
(Pro)  
TATCACAGCGTGTCTCATGCCCGCGCGCGCTGGATCTGGTTTGCCTACTCCTGCTTGCT  
ATAGTGCCACAGAGTACGGGCGGGGCGACCTAGACCAAAACGGATGAGGACGAACGA

FIG. 1X

25 / 26

## FIG. 1Y

9001 AlaGlyValGlyIleTyrLeuLeuProAsnArgOP  
GCAGGGGTAGGCATCTACCTCCTCCCAACCGATGAAGTTGGGTAAACACTCCGGCCT  
CGTCCCATCCGTAGATGGAGGGGTTGGCTACTTCCCAACCCCATTTGTGAGGCCGGA

( ) = Heterogeneity due possibly to 5' or 3'-  
terminal cloning artefact

26 / 26

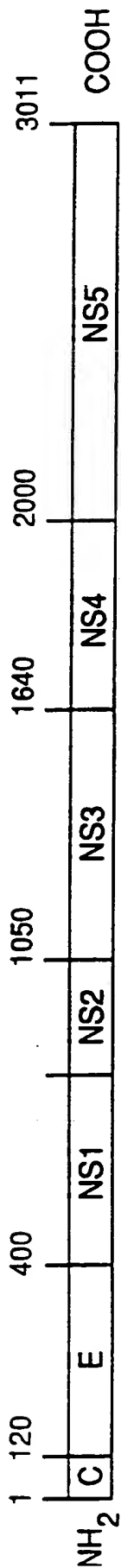


FIG. 2

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/02225

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>5</sup> : G 01 N 33/576, C 07 K 15/00														
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; font-size: small;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border: none;"> <tr> <td style="width: 20%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Classification System         </div> <div style="border: 1px solid black; padding: 5px;">             IPC<sup>5</sup> </div> </td> <td style="width: 80%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Classification Symbols         </div> <div style="border: 1px solid black; padding: 5px;">             G 01 N, C 07 K           </div> </td> </tr> </table> <div style="text-align: center; font-size: x-small; margin-top: 5px;">       Documentation Searched other than Minimum Documentation        to the Extent that such Documents are Included in the Fields Searched *     </div>			<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Classification System         </div> <div style="border: 1px solid black; padding: 5px;">             IPC<sup>5</sup> </div>	<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Classification Symbols         </div> <div style="border: 1px solid black; padding: 5px;">             G 01 N, C 07 K           </div>										
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<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT *</b> <table style="width: 100%; border: none;"> <tr> <th style="width: 10%; border: none; font-size: x-small;">Category *</th> <th style="width: 70%; border: none; font-size: x-small;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; border: none; font-size: x-small;">Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="border: none; padding: 5px;">           WO, A, 8904669 (CHIRON CORP.)            1 June 1989            see page 39, lines 8-12; page 49, line            5 - page 50, line 31; page 123, line            29 - page 125, line 22; page 132, line            3 - page 134, line 35; page 171,            lines 4-20            --         </td> <td style="border: none; text-align: center; vertical-align: top; padding: 5px;">1-16</td> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="border: none; padding: 5px;">           EP, A, 0318216 (CHIRON CORP.)            31 May 1989            see page 15, line 39 - page 17, line            8; page 18, line 44 - page 19, line            13; page 27, lines 10-22            cited in the application            --         </td> <td style="border: none; text-align: center; vertical-align: top; padding: 5px;">1-16</td> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="border: none; padding: 5px;">           Science, vol. 244, 21 April 1989,            (Washington, DC, US),            G. Kuo et al.: "An assay for circula-            ting antibodies to a major etiologic            virus of human non-A, non-B hepatitis",            pages 362-364            see the whole article            -----         </td> <td style="border: none; text-align: center; vertical-align: top; padding: 5px;">1-16</td> </tr> </table>			Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	Y	WO, A, 8904669 (CHIRON CORP.) 1 June 1989 see page 39, lines 8-12; page 49, line 5 - page 50, line 31; page 123, line 29 - page 125, line 22; page 132, line 3 - page 134, line 35; page 171, lines 4-20 --	1-16	Y	EP, A, 0318216 (CHIRON CORP.) 31 May 1989 see page 15, line 39 - page 17, line 8; page 18, line 44 - page 19, line 13; page 27, lines 10-22 cited in the application --	1-16	A	Science, vol. 244, 21 April 1989, (Washington, DC, US), G. Kuo et al.: "An assay for circula- ting antibodies to a major etiologic virus of human non-A, non-B hepatitis", pages 362-364 see the whole article -----	1-16
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<div style="display: flex; justify-content: space-between; font-size: x-small;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>														
<b>IV. CERTIFICATION</b> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Date of the Actual Completion of the International Search         </div> <div style="border: 1px solid black; padding: 5px;">             5th July 1991           </div> </td> <td style="width: 50%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Date of Mailing of this International Search Report         </div> <div style="border: 1px solid black; padding: 5px;">             - 2. 09. 91           </div> </td> </tr> <tr> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           International Searching Authority         </div> <div style="border: 1px solid black; padding: 5px;">             EUROPEAN PATENT OFFICE           </div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Signature of Authorized Officer         </div> <div style="border: 1px solid black; padding: 5px; display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 10px; margin-right: 10px;">M. PEIS</div> <div style="font-family: cursive; font-size: 1.2em;">M. Peis</div> </div> </td> </tr> </table>			<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Date of the Actual Completion of the International Search         </div> <div style="border: 1px solid black; padding: 5px;">             5th July 1991           </div>	<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Date of Mailing of this International Search Report         </div> <div style="border: 1px solid black; padding: 5px;">             - 2. 09. 91           </div>	<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           International Searching Authority         </div> <div style="border: 1px solid black; padding: 5px;">             EUROPEAN PATENT OFFICE           </div>	<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;">           Signature of Authorized Officer         </div> <div style="border: 1px solid black; padding: 5px; display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 10px; margin-right: 10px;">M. PEIS</div> <div style="font-family: cursive; font-size: 1.2em;">M. Peis</div> </div>								
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**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9102225  
SA 46573

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The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8904669	01-06-89	AU-A- 2796789	14-06-89
		EP-A- 0318216	31-05-89
		GB-A- 2212511	26-07-89
		JP-T- 2500880	29-03-90
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EP-A- 0318216	31-05-89	AU-A- 2796789	14-06-89
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